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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Use of clusterin for the treatment and/or prevention of peripheral neurological diseases

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USE OF CLUSTERIN FOR THE TREATMENT AND/OR PREVENTION OF PERIPHERAL NEUROLOGICAL DISEASES

FIELD OF THE INVENTION

The present invention is generally in the field of neurological diseases of the peripheral nervous system. It relates to neuroprotection, nerve myelination and generation or re-generation of myelin producing cells. More specifically, the present invention relates to the use of clusterin, or of an agonist of clusterin activity, for the manufacture of a medicament for treatment and/or prevention of a peripheral neurological disease.

BACKGROUND OF THE INVENTION

Peripheral neurological diseases are disorders relating to the peripheral nervous system (PNS) or the peripheral glia supporting the PNS. Peripheral neuropathies are among the most common peripheral neurological diseases.

Peripheral Neuropathy is a syndrome of sensory loss, muscle weakness and atrophy, decreased deep tendon reflexes, and vasomotor symptoms, alone or in any combination.

The disease may affect a single nerve (mononeuropathy), two or more nerves in separate areas (multiple mononeuropathy), or many nerves simultaneously (polyneuropathy). The axon may be primarily affected (e.g. in diabetes mellitus, Lyme disease, or uremia or with toxic agents) or the myelin sheath or Schwann cell (e.g. in acute or chronic inflammatory polyneuropathy, leukodystrophies, or Guillain-Barré syndrome). Damage to small unmyelinated and myelinated fibers results primarily in loss of temperature and pain sensation; damage to large myelinated fibers results in motor or proprioceptive defects. Some neuropathies (e.g. due to lead toxicity, dapsone use, tick bite, porphyria, or Guillain - Barré syndrome) primarily affect motor fibers; others (e.g. due to dorsal root ganglionitis of cancer, leprosy, AIDS, diabetes mellitus, or chronic pyridoxine intoxication) primarily affect the dorsal root ganglia or sensory fibers, producing sensory symptoms. Occasionally, cranial nerves are also involved (e.g. in Guillain - Barré syndrome, Lyme disease, diabetes mellitus, and diphtheria). Identifying the modalities involved helps determine the cause.

Trauma is the most common cause of a localized injury to a single nerve. Violent muscular activity or forcible overextension of a joint may produce a focal

neuropathy, as may, repeated small traumas (e.g. tight gripping of small tools, excessive vibration from air hammers). Pressure or entrapment paralysis usually affects superficial nerves (ulnar, radial, peroneal) at bony prominences (e.g. during sound sleep or during anesthesia in thin or cachectic persons and often in alcoholics) or at narrow canals (e.g. in carpal tunnel syndrome). Pressure paralysis may also result from tumors, bony hyperostosis, casts, crutches, or prolonged cramped postures (e.g. in gardening). Hemorrhage into a nerve and exposure to cold or radiation may cause neuropathy. Mononeuropathy may result from direct tumor invasion.

Traumatic nerve injury of the PNS can be caused during surgery (e.g. surgical prostatectomy). In nerve-sparing prostatectomy, in order to avoid nerve damage, the practice is the stimulation of the cavernous nerve during surgery to identify the course of cavernous nerves and guide the surgeon in avoiding nerve damage (Klotz and Herschorn, 1998). Studies assessing the outcome of impotency following radical prostatectomy demonstrated 212 of 503 previously potent men (42%) suffered impotency when partial or complete resection of one or both cavernosal nerve(s). This impotency rate decreased to 24% when the nerves were left intact (Quinlan et al., Feb 199; May 1991).

Multiple mononeuropathy is usually secondary to collagen vascular disorders (e.g. polyarteritis nodosa, SLE, Sjögren's syndrome, RA), sarcoidosis, metabolic diseases (e.g. diabetes, amyloidosis), or infectious diseases (e.g. Lyme disea se, HIV infection). Microorganisms may cause multiple mononeuropathy by direct invasion of the nerve (e.g. in leprosy).

Polyneuropathy due to acute febrile diseases may result from a toxin (e.g. in diphtheria) or an autoimmune reaction (e.g. in Guillain-Barré syndrome); the polyneuropathy that sometimes follows immunizations is probably also autoimmune.

Toxic agents generally cause polyneuropathy but sometimes mononeuropathy. They include emetine, hexobarbital, barbital, chlorobutanol, sulfonamides, phenytoin, nitrofurantoin, the vinca alkaloids, heavy metals, carbon monoxide, triorthocresyl phosphate, orthodinitrophenol, many solvents, other industrial poisons, and certain AIDS drugs (e.g. zalcitabine, didanosine).

Nutritional deficiencies and metabolic disorders may result in polyneuropathy. B vitamin deficiency is often the cause (e.g. in alcoholism, beriberi, pernicious anemia, isoniazid-induced pyridoxine deficiency, malabsorption syndromes, and hyperemesis gravidarum). Polyneuropathy also occurs in hypothyroidism, porphyria, sarcoidosis, amyloidosis, and uremia. Diabetes mellitus

can cause sensorimotor distal polyneuropathy (most common), multiple mononeuropathy, and focal mononeuropathy (e.g. of the oculomotor or abducens cranial nerves).

Malignancy may cause polyneuropathy via monoclonal gammopathy (multiple myeloma, lymphoma), amyloid invasion, or nutritional deficiencies or as a paraneoplastic syndrome.

Specific mononeuropathies: Single and multiple mononeuropathies are characterized by pain, weakness, and paresthesias in the distribution of the affected nerve. Multiple mononeuropathy is asymmetric; the nerves may be involved all at once or progressively. Extensive involvement of many nerves may simulate a polyneuropathy.

Ulnar nerve palsy is often caused by trauma to the nerve in the ulnar groove of the elbow by repeated leaning on the elbow or by asymmetric bone growth after a childhood fracture (tardy ulnar palsy). The ulnar nerve can also be compressed at the cubital tunnel. Paresthesias and a sensory deficit in the 5th and medial half of the 4th fingers occur; the thumb adductor, 5th finger abductor, and interossei muscles are weak and atrophied. Severe chronic ulnar palsy produces a clawhand deformity. Nerve conduction studies can identify the site of the lesion. Conservative treatment should be attempted before surgical repair is attempted.

The carpal tunnel syndrome results from compression of the median nerve in the volar aspect of the wrist between the transverse superficial carpal ligament and the longitudinal tendons of forearm muscles that flex the hand. It may be unilateral or bilateral. The compression produces paresthesias in the radial-palmar aspect of the hand and pain in the wrist and palm; sometimes pain occurs proximally to the compression site in the forearm and shoulder. Pain may be more severe at night. A sensory deficit in the palmar aspect of the first three fingers may follow; the muscles that control thumb abduction and opposition may become weak and atrophied. This syndrome should be distinguished from C-6 root compression due to cervical radiculopathy.

Peroneal nerve palsy is usually caused by compression of the nerve against the lateral aspect of the fibular neck. It is most common in emaciated bedridden patients and in thin persons who habitually cross their legs. Weakness of foot dorsiflexion and eversion (footdrop) occur. Occasionally, a sensory deficit occurs over the anterolateral aspect of the lower leg and dorsum of the foot or in the web space between the 1st and 2nd metatarsals. Treatment is usually conservative for compressive neuropathies (e.g. avoiding leg crossing). Incomplete neuropathies are

usually followed clinically and usually improve spontaneously. If recovery does not occur, surgical exploration may be indicated.

Radial nerve palsy (Saturday night palsy) is caused by compression of the nerve against the humerus, e.g. as the arm is draped over the back of a chair during intoxication or deep sleep. Symptoms include weakness of wrist and finger extensors (wristdrop) and, occasionally, sensory loss over the dorsal aspect of the 1st dorsal interosseous muscle. Treatment is similar to that of compressive peroneal neuropathy.

Polyneuropathies are relatively symmetric, often affecting sensory, motor, and vasomotor fibers simultaneously. They may affect the axon cylinder or the myelin sheath and, in either form, may be acute (e.g. Guillain-Barré syndrome) or chronic (e.g. renal failure).

Polyneuropathy due to metabolic disorders (e.g. diabetes mellitus) or renal failure develops slowly, often over months or years. It frequently begins with sensory abnormalities in the lower extremities that are often more severe distally than proximally. Peripheral tingling, numbness, burning pain, or deficiencies in joint proprioception and vibratory sensation are often prominent. Pain is often worse at night and may be aggravated by touching the affected area or by temperature changes. In severe cases, there are objective signs of sensory loss, typically with stocking-and-glove distribution. Achilles and other deep tendon reflexes are diminished or absent. Painless ulcers on the digits or Charcot's joints may develop when sensory loss is profound. Sensory or proprioceptive deficits may lead to gait abnormalities. Motor involvement results in distal muscle weakness and atrophy. The autonomic nervous system may be additionally or selectively involved, leading to nocturnal diarrhea, urinary and fecal incontinence, impotence, or postural hypotension. Vasomotor symptoms vary. The skin may be paler and drier than normal, sometimes with dusky discoloration; sweating may be excessive. Trophic changes (smooth and shiny skin, pitted or ridged nails, osteoporosis) are common in severe, prolonged cases.

Nutritional polyneuropathy is common among alcoholics and the malnourished. A primary axonopathy may lead to secondary demyelination and axonal destruction in the longest and largest nerves. Whether the cause is deficiency of thiamine or another vitamin (e.g. pyridoxine, pantothenic acid, folic acid) is unclear. Neuropathy due to pyridoxine deficiency usually occurs only in persons taking isoniazid for TB; infants who are deficient or dependent on pyridoxine may have convulsions. Wasting and symmetric weakness of the distal

extremities is usually insidious but can progress rapidly, sometimes accompanied by sensory loss, paresthesias, and pain. Aching, cramping, coldness, burning, and numbness in the calves and feet may be worsened by touch. Multiple vitamins may be given when etiology is obscure, but they have no proven benefit.

Uncommonly, an exclusively sensory polyneuropathy begins with peripheral pains and paresthesias and progresses centrally to a loss of all forms of sensation. It occurs as a remote effect of carcinoma (especially bronchogenic), after excessive pyridoxine ingestion (> 0.5 g/day), and in amyloidosis, hypothyroidism, myeloma, and uremia. The pyridoxine-induced neuropathy resolves when pyridoxine is discontinued.

Hereditary neuropathies are classified as sensorimotor neuropathies or sensory neuropathies. Charcot-Marie-Tooth disease is the most common hereditary sensorimotor neuropathy. Less common sensorimotor neuropathies begin at birth and result in greater disability. In sensory neuropathies, which are rare, loss of distal pain and temperature sensation is more prominent than loss of vibratory and position sense. The main problem is pedal mutilation due to pain insensitivity, with frequent infections and osteomyelitis.

Hereditary motor and sensory neuropathy types I and II (Charcot-Marie-Tooth disease, peroneal muscular atrophy) is a relatively common, usually autosomal dominant disorder characterized by weakness and atrophy, primarily in peroneal and distal leg muscles. Patients may also have other degenerative diseases (e.g. Friedreich's ataxia) or a family history of them. Patients with type I present in middle childhood with footdrop and slowly progressive distal muscle atrophy, producing "stork legs." Intrinsic muscle wasting in the hands begins later. Vibration, pain, and temperature sensation decreases in a stocking-glove pattern. Deep tendon reflexes are absent. High pedal arches or hammer toes may be the only signs in less affected family members who carry the disease. Nerve conduction velocities are slow, and distal latencies prolonged. Segmental demyelination and remyelination occur. Enlarged peripheral nerves may be palpated. The disease progresses slowly and does not affect life span. Type II disease evolves more slowly, with weakness usually developing later in life. Patients have relatively normal nerve conduction velocities but low amplitude evoked potentials. Biopsies show wallerian degeneration.

Hereditary motor and sensory neuropathy type III (hypertrophic interstitial neuropathy, Dejerine-Sottas disease), a rare autosomal recessive disorder, begins in childhood with progressive weakness and sensory loss and absent deep tendon

reflexes. Initially, it resembles Charcot-Marie-Tooth disease, but motor weakness progresses at a faster rate. Demyelination and remyelination occur, producing enlarged peripheral nerves and onion bulbs seen on nerve biopsy.

The characteristic distribution of motor weakness, foot deformities, family history, and electrophysiologic abnormalities confirm the diagnosis. Genetic analysis is available, but no specific treatment. Vocational counseling to prepare young patients for disease progression may be useful. Bracing helps correct footdrop; orthopedic surgery to stabilize the foot may help.

Spinal cord injuries account for the majority of hospital admissions for paraplegia and tetraplegia. Over 80% occur as a result of road accidents. Two main groups of injury are recognised clinically: open injuries and closed injuries.

Open injuries cause direct trauma of the spinal cord and nerve roots. Perforating injuries can cause extensive disruption and hemorrhage. Closed injuries account for most spinal injuries and are usually associated with a fracture/dislocation of the spinal column, which is usually demonstrable radiologically. Damage to the cord depends on the extent of the bony injuries and can be considered in two main stages: Primary damage, which are contusions, nerve fiber transections and hemorrhagic necrosis, and secondary damage, which are extradural heamatoma, infarction, infection and edema.

Late effects of cord damage include: ascending and descending anterograde degeneration of damaged nerve fibers, post-traumatic syringomelyia, and systemic effects of paraplegia, such as urinary tract and chest infections, pressure sores and muscle wasting.

Demyelination is linked to functional reduction or blockage in neural impulse conduction.

The multilamellar myelin sheath is a specialized domain of the glial cell plasma membrane, rich in lipid and low in protein. It serves to support axons and improve the efficiency of electrical signal conduction in the nervous system by preventing the charge from bleeding off into the surrounding tissue. The nodes of Ranvier are the sites in the sheath along the axon where saltatory conductance occurs.

The process of remyelination could work in concert with anti-inflammatory pathways to repair damage and protect axons from transection and death.

Schwann cells are peripheral glia cells providing a supportive role in the peripheral nervous system and belong to the satellite cells. Schwann cells wrap individually around the shaft of peripheral axons, forming a layer or myelin sheath

along segments of the axon. Schwann cells are composed primarily of lipids or fats; the fat serves as an insulator thereby speeding the transmission rate of action potentials along the axon.

Schwann cells are also essential to the process of neuronal regeneration in the peripheral nervous system. When an axon is dying, the Schwann cells surrounding it aid in its digestion. This leaves an empty channel formed by successive Schwann cells, through which a new axon may grow from a severed end at a rate of 3-4 millimeters a day.

Clusterin is an extracellular protein that is also known as Apolipoprotein J, SGP-2, TRPM-2 and SP-40,40. It has a nearly ubiquitous tissue distribution and many names have been given to it according to the source where it was purified (reviewed in Trougakos and Gonos 2002, Jones and Jomary 2002). Despite its ubiquitous expression and its relative abundance of serum (100ug/ml) the genuine function of clusterin remains unraveled. Several biological roles of clusterin have been proposed among which the ability to inhibit complement cascade by binding C9 complement (Tschopp et al., 1993), a pro-apoptotic activity or an anti-apoptotic activity depending on animal models studied (Han et al. 2001, Wehrli et al, 2001), limitation of progression and more recently chaperone properties (Poon et al., 2002). A neuroprotective role of clusterin in Alzheimer's disease has also been suggested (Giannakopoulos, 1998). Its major form, a 75-80 kDa heterodimer is issued from a single transcript. The polypeptide chain is then cleaved proteolytically to remove the 22-mer secretory signal peptide and subsequently between residues 227/228 to generate two chains, alpha and beta, that are assembled by 5 cysteine-bonds located in the center of each chain. The polypeptide also contains glycosylation sites and nuclear localization signals sequences. Its degradation seems to be mediated by the endocytic receptor gp330/megalin/LRP2 a member of the low-density lipoprotein receptor family (Kounnas et al., 1995).

Interferons are a subclass of cytokines that exhibit anti-inflammatory, antiviral and anti-proliferative activity. On the basis of biochemical and immunological properties, the naturally-occurring human interferons are grouped into three classes: interferon alpha (leukocyte), interferon beta (fibroblast) and interferon gamma (immune). Alpha-interferon is currently approved in the United States and other countries for the treatment of hairy cell leukemia, venereal warts, Kaposi's Sarcoma (a cancer commonly afflicting patients suffering from Acquired Immune Deficiency Syndrome (AIDS)), and chronic non-A, non-B hepatitis.

Further, interferons (IFNs) are glycoproteins produced by the body in response to a viral infection. They inhibit the multiplication of viruses in protected cells. Consisting of a lower molecular weight protein, IFNs are remarkably non-specific in their action, i.e. IFN induced by one virus is effective against a broad range of other viruses. They are however species-specific, i.e. IFN produced by one species will only stimulate antiviral activity in cells of the same or a closely related species. IFNs were the first group of cytokines to be exploited for their potential antitumour and antiviral activities.

The three major IFNs are referred to as IFN-α, IFN-β and IFN-γ. Such main kinds of IFNs were initially classified according to their cells of origin (leukocyte, fibroblast or T cell). However, it became clear that several types might be produced by one cell. Hence leukocyte IFN is now called IFN-α, fibroblast IFN is IFN-β and T cell IFN is IFN-γ. There is also a fourth type of IFN, lymphoblastoid IFN, produced in the "Namalwa" cell line (derived from Burkitt's lymphoma), which seems to produce a mixture of both leukocyte and fibroblast IFN.

The Interferon unit has been reported as a measure of IFN activity defined (somewhat arbitrarily) as the amount necessary to protect 50% of the cells against viral damage.

Every class of IFN contains several distinct types. IFN-β and IFN-γ are each the product of a single gene. The differences between individual types seem to be mainly due to variations in glycosylation.

IFNs-α are the most diverse group, containing about 15 types. There is a cluster of IFN-α genes on chromosome 9, containing at least 23 members, of which 15 are active and transcribed. Mature IFNs-α is not glycosylated.

IFNs-α and IFN-β are all the same length (165 or 166 amino acids) with similar biological activities. IFNs-γ are 146 amino acids in length, and resemble the α and β classes less closely. Only IFNs-γ can activate macrophages or induce the maturation of killer T cells. In effect, these new types of therapeutic agents can be called biologic response modifiers (BRMs), because they have an effect on the response of the organism to the tumour, affecting recognition via immunomodulation.

In particular, human fibroblast interferon (IFN-β) has antiviral activity and can also stimulate natural killer cells against neoplastic cells. It is a polypeptide of about 20,000 Da induced by viruses and double-stranded RNAs. From the nucleotide sequence of the gene for fibroblast interferon, cloned by recombinant DNA

technology, Derynk et al. (Derynk R. et al, 1980) deduced the complete amino acid sequence of the protein. It is 166 amino acid long.

Shepard et al. (Shepard H. M. et al, 1981) described a mutation at base 842 (Cys → Tyr at position 141) that abolished its anti-viral activity, and a variant clone with a deletion of nucleotides 1119-1121.

Mark et al. (Mark et al, 1984) inserted an artificial mutation by replacing base 469 (T) with (A) causing an amino acid switch from Cys → Ser at position 17. The resulting IFN-β was reported to be as active as the 'native' IFN-β and stable during long-term storage (-70°C).

The mechanisms by which IFNs exert their effects are not completely understood. However, in most cases they act by affecting the induction or transcription of certain genes, thus affecting the immune system. In vitro studies have shown that IFNs are capable of inducing or suppressing about 20 gene products.

Osteopontin (OPN) is a highly phosphorylated sialoprotein that is a prominent component of the mineralized extracellular matrices of bo nes and teeth. OPN is characterized by the presence of a polyaspartic acid sequence and sites of Ser/Thr phosphorylation that mediate hydroxyapatite binding, and a highly conserved RGD motif that mediates cell attachment/signalling. Osteopontin inhibitors have been described said to be useful for treatment of infections, immune disorders and diseases, autoimmune disorders, including MS, various immunodeficiencies, and cancer, WO 00/63241.

SUMMARY OF THE INVENTION

It is the object of the present invention to provide novel means for the treatment and/or prevention of peripheral neurological diseases.

The invention is based on the finding that the protein clusterin has a beneficial effect in an animal model of peripheral neuropathy.

Therefore, the present invention relates to the use of clusterin, or of an agonist of clusterin activity, in a peripheral neurological disease, such as traumatic nerve injury of the peripheral nervous system (PNS), and peripheral neuropathies.

The use of nucleic acid molecules, and expression vectors comprising clusterin, and of cells expressing clusterin, for treatment and/or prevention of a peripheral neurological disease is also within the present invention.

The invention further provides pharmaceutical compositions comprising clusterin and an interferon or osteopontin, optionally together with one or more pharmaceutically acceptable excipients.

In a second aspect of the invention, clusterin may be used in combination with an interferon or osteopontin for treatment and/or prevention of peripheral neurological diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 schematically depicts the structure of clusterin. (A) is the precursor polypeptide, (B) is a representation of the mature polypeptide, which is a heterodimeric glycoprotein of 75-80 kDa formed by an α (34-36 kDa) and β (36-39 kDa) chain linked in antiparallel by 5 disulfide bridges near their centers, (C) shows the sequence of human clusterin precursor.

Fig. 2 schematically depicts the plasmid pEAK12 DEST comprising the clusterin coding sequence with a HIS-Tag.

Fig. 3 shows the body weight of neuropathic mice induced by sciatic nerve crush treated with vehicle, 300 µg/kg or 1 mg/kg of AS900035 (clusterin) administered intraperitoneally (i.p.). Control: healthy mice.

Fig. 4 shows the amplitude of the compound muscle action potential in neuropathic mice treated with vehicle, 300 µg/kg or 1mg/kg i.p. of AS 900035 (clusterin), 0.01 µg/kg of a positive control compound (4-MC) or 100 µg/kg subcutaneous (s.c.) of AS900011 (osteopontin). Control: sham operated mice.

Fig. 5 shows the latency of the compound muscle action potential in neuropathic mice treated with vehicle, 300 µg/kg or 1mg/kg i.p. of AS900035 (clusterin), 0.01 µg/kg of a positive control compound (4-MC) or 100 µg/kg s.c. of AS900011 (osteopontin). Control: sham operated mice.

Fig. 6 shows the duration of the compound muscle action potential in the neuropathic mice treated with vehicle, 300 µg/kg or 1mg/kg i.p. of AS900035 (clusterin), 0.01 µg/kg of a positive control compound (4-MC) or 100 µg/kg s.c. of AS900011 (osteopontin). Control: sham operated mice.

Fig. 7 shows total number of degenerated fibers in the neuropathic mice treated with vehicle, 300 µg/kg, or 1mg/kg i.p. of AS900035 (clusterin). Control: sham operated mice.

Fig. 8 shows the percentage of non-degenerated fibers in the neuropathic mice treated with vehicle, 300 µg/kg, or 1mg/kg of AS900035 (clusterin). Control: sham operated mice.

DETAILED DESCRIPTION OF THE INVENTION

In the frame of the present invention, it has been found that administration of clusterin has a beneficial effect in an in vivo animal model of peripheral neurological disease. In a murine model of sciatic nerve crush induced neuropathy, all physiologic and morphologic parameters relating to nerve regeneration, integrity and vitality were positively influenced by administration of clusterin.

The invention therefore relates to the use of clusterin, an isoform, mutein, fused protein, functional derivative, active fraction, circularl y permutated derivative, or salt thereof, or of an agonist of clusterin activity, for the manufacture of a medicament for treatment and/or prevention of peripheral neurological diseases.

The term "clusterin", as used herein, relates to full-length mature human clusterin, or to any of the clusterin subunits, or a fragment thereof. The sequence of human clusterin is reported herein as SEQ ID NO: 1 of the annexed sequence listing, and in Fig. 1C of the annexed drawings. The term "clusterin", as used herein, further relates to any clusterin derived from animals, such as murine, bovine, porcine, feline or ovine clusterin, as long as there is sufficient identity in order to maintain clusterin activity, and as long as the resulting molecule will not be immunogenic in humans.

The term "clusterin", as used herein, further relates to biologically active muteins and fragments, such as the naturally occurring alpha and beta subunit of clusterin.

The term "clusterin", as used herein, further encompasses isoforms, muteins, fused proteins, functional derivatives, active fractions or fragments, or circularly permutated derivatives, or salts thereof. These isoforms, muteins, fused proteins or functional derivatives, active fractions or fragments, or circularly permutated

derivatives retain the biological activity of clusterin. Preferably, they have a biological activity, which is improved as compared to wild type clusterin.

The term "agonist of clusterin activity", as used herein, relates to a molecule stimulating or mimicking clusterin activity, such as agonistic antibodies of a clusterin receptor, or small molecular weight agonists activating signaling through a clusterin receptor. A clusterin receptor maybe e.g. gp330/megalin/LRP2 (Kounnas et al., 1995). Any agonist, stimulator or enhancer, of such a receptor is encompassed by the term "agonist of clusterin activity", as used herein.

The term "agonist of clusterin activity", as used herein, further refers to agents enhancing clusterin mediated activities, such as small molecular weight compounds mimicking the clusterin activity.

The terms "treating" and "preventing", as used herein, should be understood as preventing, inhibiting, attenuating, ameliorating or reversing one or more symptoms or cause(s) of peripheral neurological diseases, as well as symptoms, diseases or complications accompanying peripheral neurological disease. When "treating" peripheral neurological disease, the substances according to the invention are given after onset of the disease, "prevention" relates to administration of the substances before signs of disease can be noted in the patient.

The term "peripheral neurological diseases", as used herein encompasses all known peripheral neurological diseases or disorders, or injuries of the PNS, including those described in detail in the "Background of the invention".

Peripheral neurological diseases comprise disorders linked to dysfunction of the PNS, such as diseases related to neurotransmission, nerve trauma, PNS infections, demyelinating diseases of the PNS, or neuropathies of the PNS.

Preferably, the peripheral neurological diseases of the invention are selected from the group consisting of traumatic nerve injury of the peripheral nervous system, demyelinating diseases of the PNS, and peripheral neurodegenerative diseases and peripheral neuropathies.

Traumatic nerve injury may concern the PNS as described in the "Background of the invention" above.

Peripheral neuropathy may be related to a syndrome of sensory loss, muscle weakness and atrophy, decreased deep tendon reflexes, and vasomotor symptoms, alone or in any combination. They may e.g. be due to alcoholism, diabetes or chemotherapeutic treatment.

Neuropathy may affect a single nerve (mononeuropathy), two or more nerves in separate areas (multiple mononeuropathy), or many nerves

simultaneously (polyneuropathy). The axon may be primarily affected (e.g. in diabetes mellitus, Lyme disease, or uremia or with toxic agents), or the myelin sheath or Schwann cell (e.g. in acute or chronic inflammatory polyneuropathy, leukodystrophies, or Guillain-Barré syndrome). Further neuropathies, which may be treated in accordance with the present invention, may e.g. be due to lead toxicity, dapsone use, tick bite, porphyria, or Guillain-Barré syndrome, and they may primarily affect motor fibers. Others, such as those due to dorsal root ganglionitis of cancer, leprosy, AIDS, diabetes mellitus, or chronic pyridoxine intoxication, may primarily affect the dorsal root ganglia or sensory fibers, producing sensory symptoms. Cranial nerves may also be involved, such as e.g. in Guillain-Barré syndrome, Lyme disease, diabetes mellitus, and diphtheria.

Further peripheral neurological disorders comprise neuropathies with abnormal myelination, such as the ones listed in the "Background of the invention" above, as well as carpal tunnel syndrome. Traumatic nerve injury may be accompanied by spinal column orthopedic complications, and those are also within the diseases in accordance with the present invention.

Peripheral neurological disorders may further be due to congenital metabolic disorders. In a preferred embodiment of the invention, the peripheral neurological disease is therefore due to a congenital metabolic deficit.

In a further preferred embodiment, the peripheral neurological disease is a peripheral neuropathy, most preferably diabetic neuropathy. Chemotherapy associated neuropathies are also preferred in accordance with the present invention.

The term "diabetic neuropathy" relates to any form of diabetic neuropathy, or to one or more symptom(s) or disorder(s) accompanying or caused by diabetic neuropathy, or complications of diabetes affecting nerves as described in detail in the "Background of the invention" above. Diabetic neuropathy may be a polyneuropathy. In diabetic polyneuropathy, many nerves are simultaneously affected. The diabetic neuropathy may also be a mononeuropathy. In focal mononeuropathy, for instance, the disease affects a single nerve, such as the oculomotor or abducens cranial nerve. It may also be multiple mononeuropathy when two or more nerves are affected in separate areas.

In yet a further preferred embodiment, the peripheral neurological disorder is a demyelinating disease of the peripheral nervous system (PNS). The latter comprise diseases such as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and acute, monophasic disorders, such as the

inflammatory demyelinating polyradiculoneuropathy termed Guillain -Barré syndrome (GBS).

Preferably, the clusterin is selected from a peptide, a polypeptide or a protein selected from the group consisting of:

a) A polypeptide comprising SEQ ID NO: 1;
b) A polypeptide comprising amino acids 23 to 449 of SEQ ID NO: 1;
c) A polypeptide comprising amino acids 35 to 449 of SEQ ID NO: 1;
d) A polypeptide comprising amino acids 23 to 227 of SEQ ID NO: 1;
e) A polypeptide comprising amino acids 35 to 227 of SEQ ID NO: 1;
f) A polypeptide comprising amino acids 228 to 449 of SEQ ID NO: 1;
g) A mutein of any of (a) to (f), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (f);
h) A mutein of any of (a) to (f) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (f) under moderately stringent conditions or under highly stringent conditions;
i) A mutein of any of (a) to (f) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (f);
j) a salt or an isoform, fused protein, functional derivative, active fraction or circularly permutated derivative of any of (a) to (f).

Active fractions or fragments may comprise any portion or domain of clusterin, such as the alpha chain or the beta chain separated, or linked to each other e.g. via di-sulfide bridges, directly fused, or fused via an appropriate linker. Active fractions also comprise differentially glycosylated or sialylated forms of clusterin.

The person skilled in the art will appreciate that even smaller portions of clusterin or its two subunits may be enough to exert its function, such as an active peptide comprising the essential amino acid residues required for clusterin function.

The person skilled in the art will further appreciate that muteins, salts, isoforms, fused proteins, functional derivatives of clusterin, active fractions or circularly permutated derivatives of clusterin, will retain a similar, or even better, biological activity of clusterin. The biological activity of clusterin and muteins,

isoforms, fused proteins or functional derivatives, active fractions or fragments, circularly permutated derivatives, or salts thereof, may be measured in a co-culturing assay.

Preferred active fractions have an activity which is equal or better than the activity of full-length clusterin, or which have further advantages, such as a better stability or a lower toxicity or immunogenicity, or they are easier to produce in large quantities, or easier to purify. The person skilled in the art will appreciate that muteins, active fragments and functional derivatives can be generated by cloning the corresponding cDNA in appropriate plasmids and testing them in the co-culturing assay, as mentioned above.

The proteins according to the present invention may be glycosylated or non-glycosylated, they may be derived from natural sources, such as body fluids, or they may preferably be produced recombinantly. Recombinant expression may be carried out in prokaryotic expression systems such as E. coli, or in eukaryotic, such as insect cells, and preferably in mammalian expression systems, such as CHO-cells or HEK-cells.

As used herein the term "muteins" refers to analogs of clusterin, in which one or more of the amino acid residues of a natural clusterin are replaced by different amino acid residues, or are deleted, or one or more amino acid residues are added to the natural sequence of clusterin, without changing considerably the activity of the resulting products as compared with the wild-type clusterin. These muteins are prepared by known synthesis and/or by site-directed mutagenesis techniques, or any other known technique suitable therefore.

Muteins of clusterin, which can be used in accordance with the present invention, or nucleic acid coding thereof, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein.

Muteins in accordance with the present invention include proteins encoded by a nucleic acid, such as DNA or RNA, which hybridizes to DNA or RNA, which encodes clusterin, in accordance with the present invention, under moderately or highly stringent conditions. The term "stringent conditions" refers to hybridization and subsequent washing conditions, which those of ordinary skill in the art conventionally refer to as "stringent". See Ausubel et al., Current Protocols in Molecular Biology, supra, Interscience, N.Y., §§6.3 and 6.4 (1987, 1992), and Sambrook et al. (Sambrook, J. C., Fritsch, E. F., and Maniatis, T. (1989) Molecular

Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Without limitation, examples of stringent conditions include washing conditions 12-20°C below the calculated Tm of the hybrid under study in, *e.g.*, 2 x SSC and 0.5% SDS for 5 minutes, 2 x SSC and 0.1% SDS for 15 minutes; 0.1 x SSC and 0.5% SDS at 37°C for 30-60 minutes and then, a 0.1 x SSC and 0.5% SDS at 68°C for 30-60 minutes. Those of ordinary skill in this art understand that stringency conditions also depend on the length of the DNA sequences, oligonucleotide probes (such as 10-40 bases) or mixed oligonucleotide probes. If mixed probes are used, it is preferable to use tetramethyl ammonium chloride (TMAC) instead of SSC. See Ausubel, supra.

In a preferred embodiment, any such mutein has at least 40% identity or homology with the sequence of SEQ ID NO: 1 of the annexed sequence listing. More preferably, it has at least 50%, at least 60%, at least 70%, at least 80% or, most preferably, at least 90% identity or homology thereto.

Identity reflects a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, determined by comparing the seq uences. In general, identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of the two polynucleotides or two polypeptide sequences, respectively, over the length of the sequences being compared.

For sequences where there is not an exact correspondence, a "% identity" may be determined. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or very similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

Methods for comparing the identity and homology of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux et al., 1984), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % homology between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (1981) and finds the best single region of similarity between two sequences. Other programs for determining identity and/or similarity between sequences are also

known in the art, for instance the BLAST family of programs (Altschul et al, 1990, Altschul et al, 1997, accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov) and FASTA (Pearson W R, 1990; Pearson 1988).

Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of clusterin polypeptides, may include synonymous amino acids within a group which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the molecule (Grantham, 1974). It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, e.g. under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e.g. cysteine residues. Proteins and muteins produced by such deletions and/or insertions come within the purview of the present invention.

Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined in Table III.

TABLE I

Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser, Thr, Gly, Asn
Arg	Arg, Gln, Lys, Glu, His
Leu	Ile, Phe, Tyr, Met, Val, Leu
Pro	Gly, Ala, Thr, Pro
Thr	Pro, Ser, Ala, Gly, His, Gln, Thr
Ala	Gly, Thr, Pro, Ala
Val	Met, Tyr, Phe, Ile, Leu, Val
Gly	Ala, Thr, Pro, Ser, Gly
Ile	Met, Tyr, Phe, Val, Leu, Ile
Phe	Trp, Met, Tyr, Ile, Val, Leu, Phe
Tyr	Trp, Met, Phe, Ile, Val, Leu, Tyr
Cys	Ser, Thr, Cys
His	Glu, Lys, Gln, Thr, Arg, His
Gln	Glu, Lys, Asn, His, Thr, Arg, Gln
Asn	Gln, Asp, Ser, Asn
Lys	Glu, Gln, His, Arg, Lys

Asp	Glu, Asn, Asp
Glu	Asp, Lys, Asn, Gln, His, Arg, Glu
Met	Phe, Ile, Val, Leu, Met
Trp	Trp

TABLE II

More Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	His, Lys, Arg
Leu	Leu, Ile, Phe, Met
Pro	Ala, Pro
Thr	Thr
Ala	Pro, Ala
Val	Val, Met, Ile
Gly	Gly
Ile	Ile, Met, Phe, Val, Leu
Phe	Met, Tyr, Ile, Leu, Phe
Tyr	Phe, Tyr
Cys	Cys, Ser
His	His, Gln, Arg
Gln	Glu, Gln, His
Asn	Asp, Asn
Lys	Lys, Arg
Asp	Asp, Asn
Glu	Glu, Gln
Met	Met, Phe, Ile, Val, Leu
Trp	Trp

TABLE III

Most Preferred Groups of Synonymous Amino Acids

Amino Acid	Synonymous Group
Ser	Ser
Arg	Arg
Leu	Leu, Ile, Met
Pro	Pro
Thr	Thr
Ala	Ala
Val	Val
Gly	Gly

Ile	Ile, Met, Leu
Phe	Phe
Tyr	Tyr
Cys	Cys, Ser
His	His
Gln	Gln
Asn	Asn
Lys	Lys
Asp	Asp
Glu	Glu
Met	Met, Ile, Leu
Trp	Met

Examples of production of amino acid substitutions in proteins which can be used for obtaining muteins of clusterin, polypeptides or proteins, for use in the present invention include any known method steps, such as presented in US patents 4,959,314, 4,588,585 and 4,737,462, to Mark et al; 5,116,943 to Koths et al., 4,965,195 to Namen et al; 4,879,111 to Chong et al; and 5,017,691 to Lee et al; and lysine substituted proteins presented in US patent No. 4,904,584 (Shaw et al).

The term "fused protein" refers to a polypeptide comprising clusterin, or a mutein or fragment thereof, fused with another protein, which e.g. has an extended residence time in body fluids. Clusterin may thus be fused to another protein, polypeptide or the like, e.g. an immunoglobulin or a fragment thereof. Immunoglobulin Fc portions are particularly suitable for production of di- or mulitmeric Ig fusion proteins. The alpha- and beta-chain of clusterin may e.g. be linked to portions of an immunoglobulin in such a way as to produce the alpha- and beta-chain of clusterin dimerized by the Ig Fc portion.

"Functional derivatives" as used herein, cover derivatives of clusterin, and their muteins and fused proteins, which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein which is substantially similar to the activity of clusterin, and do not confe r toxic properties on compositions containing it.

These derivatives may, for example, include polyethylene glycol side -chains, which may mask antigenic sites and extend the residence of clusterin in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the

carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "active fractions" of clusterin, muteins and fused proteins, the present invention covers any fragment or precursors of the polypeptid e chain of the protein molecule alone or together with associated molecules or residues linked thereto, e.g. sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said fraction has substantially si milar activity to clusterin.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of clusterin molecule or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid additi on salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Of course, any such salts must retain the biological activity of clusterin relevant to the present invention, i.e., neuroprotective effect in a peripheral neurological disease.

Functional derivatives of clusterin may be conjugated to polymers in order to improve the properties of the protein, such as the stability, half-life, bioavailability, tolerance by the human body, or immunogenicity. To achieve this goal, clusterin may be linked e.g. to Polyethlyenglycol (PEG). PEGylation may be carried out by known methods, described in WO 92/13095, for example.

Therefore, in a preferred embodiment of the present invention, clusterin is PEGylated.

In a further preferred embodiment of the invention, the fused protein comprises an immunoglobulin (Ig) fusion. The fusion may be direct, or via a short linker peptide which can be as short as 1 to 3 amino acid residues in length or longer, for example, 13 amino acid residues in length. Said linker may be a tripeptide of the sequence E-F-M (Glu-Phe-Met), for example, or a 13-amino acid linker sequence comprising Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met introduced between clusterin sequence and the immunoglobulin sequence, for

instance. The resulting fusion protein has improved properties, such as an extended residence time in body fluids (half-life), or an increased specific activity, increased expression level. The Ig fusion may also facilitate purification of the fused protein.

In a yet another preferred embodiment, clusterin or one or both subunits are fused to the constant region of an Ig molecule. Preferably, it is fused to heavy chain regions, like the CH2 and CH3 domains of human IgG1, for example. Other isoforms of Ig molecules are also suitable for the generation of fusion proteins according to the present invention, such as isoforms IgG_2 or IgG_4, or other Ig classes, like IgM, for example. Fusion proteins may be monomeric or multimeric, hetero- or homomultimeric. The immunoglobulin portion of the fused protein may be further modified in a way as to not activate complement binding or the complement cascade or bind to Fc-receptors.

The invention further relates to the use of a combination of clusterin and an immunosuppressive agent for the manufacture of a medicament for treatment and/or prevention of peripheral neurological disorders, for simultaneous, sequential or separate use. Immunosuppressive agents may be steroids, methotrexate, cyclophosphamide, anti-leukocyte antibodies (such as CAMPATH-1), and the like.

The invention further relates to the combination of clusterin and IL-6.

The invention further relates to the use of a combination of clusterin and an interferon for the manufacture of a medicament for treatment and/or prevention of peripheral neurological disorders, for simultaneous, sequential, or separate use.

The term "interferon", as used in the present patent application, is intended to include any molecule defined as such in the literature, comprising for example any kinds of IFNs mentioned in the above section "Background of the Invention". The interferon may preferably be human, but also derived from other species, as long as the biological activity is similar to human interferons, and the molecule is not immunogenic in man.

In particular, any kinds of IFN-α, IFN-β and IFN-γ are included in the above definition. IFN-β is the preferred IFN according to the present invention.

The term "interferon-beta (IFN-β)", as used in the present invention, is intended to include human fibroblast interferon, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells as well as its salts, functional derivatives, variants, analogs and fragments.

Interferons may also be conjugated to polymers in order to improve the stability of the proteins. A conjugate between Interferon β and the polyol polyethlyenglycol (PEG) has been described in WO99/55377, for instance.

In another preferred embodiment of the invention, the interferon is Interferon-β (IFN-β), and more preferably IFN-β1a.

Clusterin is preferably used simultaneously, sequentially, or separately with the interferon.

The invention further relates to the use of a combination of clusterin and osteopontin for the manufacture of a medicament for treatment and/or prevention of peripheral neurological disorders, for simultaneous, sequential, or separate use.

"Osteopontin", as used herein, encompasses also muteins, fragments, active fractions and functional derivatives of osteopontin. These proteins are described e.g. in WO 02/092122.

In a preferred embodiment of the present invention, clusterin is used in an amount of about 0.001 to 100 mg/kg of body weight, or about 1 to 10 mg/kg of body weight or about 5 mg/kg of body weight.

The invention further relates to the use of a nucleic acid molecule for manufacture of a medicament for the treatment and/or prevention of a peripheral neurological disease, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

a) A polypeptide comprising SEQ ID NO: 1;
b) A polypeptide comprising amino acids 23 to 449 of SEQ ID NO: 1;
c) A polypeptide comprising amino acids 35 to 449 of SEQ ID NO: 1;
d) A polypeptide comprising amino acids 23 to 227 of SEQ ID NO: 1;
e) A polypeptide comprising amino acids 35 to 227 of SEQ ID NO: 1;
f) A polypeptide comprising amino acids 228 to 449 of SEQ ID NO: 1;
g) A mutein of any of (a) to (f), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (e);
h) A mutein of any of (a) to (f) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (f) under moderately stringent conditions or under highly stringent conditions;
i) A mutein of any of (a) to (f) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (f); or an isoform, fused protein, functional derivative, active fraction or circularly permutated derivative of any of (a) to (f).

The nucleic acid may e.g. be administered as a naked nucleic acid molecule, e.g. by intramuscular injection.

It may further comprise vector sequences, such as viral sequence, useful for expression of the gene encoded by the nucleic acid molecule in the human body, preferably in the appropriate cells or tissues.

Therefore, in a preferred embodiment, the nucleic acid molecule further comprises an expression vector sequence. Expression vector sequences are well known in the art, they comprise further elements serving for expre ssion of the gene of interest. They may comprise regulatory sequence, such as promoter and enhancer sequences, selection marker sequences, origins of multiplication, and the like. A gene therapeutic approach is thus used for treating and/or preventing the disease. Advantageously, the expression of clusterin will then be *in situ*.

In a preferred embodiment of the invention, the expression vector may be administered by intramuscular injection.

The use of a vector for inducing and/or enhancing the endogenous production of clusterin in a cell normally silent for expression of clusterin, or which expresses amounts of clusterin which are not sufficient, are also contemplated according to the invention. The vector may comprise regulatory sequences functional in the cells desired to express clusterin. Such regulatory sequences may be promoters or enhancers, for example. The regulatory sequence may then be introduced into the appropriate locus of the genome by homologous recombination, thus operably linking the regulatory sequence with the gene, the expression of which is required to be induced or enhanced. The technology is usually referred to as "endogenous gene activation" (EGA), and it is described e.g. in WO 91/09955.

The invention further relates to the use of a cell that has been genetically modified to produce clusterin in the manufacture of a medicament for the treatment and/or prevention of peripheral neurological diseases.

The invention further relates to a cell that has been genetically modified to produce clusterin for manufacture of a medicament for the treatment and/or prevention of neurological diseases. Thus, a cell therapeutic approach may be used in order to deliver the drug to the appropriate parts of the human body.

The invention further relates to pharmaceutical compositions, particularly useful for prevention and/or treatment of peripheral neurological diseases, which comprise a therapeutically effective amount of clusterin and a therapeutically effective amount of an interferon, optionally further a therapeutically effective amount of an immuno-suppressant.

The invention further relates to pharmaceutical compositions, particularly useful for prevention and/or treatment of peripheral neurological diseases, which comprise a therapeutically effective amount of clusterin and a therapeutically effective amount of osteopontin, optionally further a therapeutically effective amount of an immuno-suppressant.

The definition of "pharmaceutically acceptable" is meant to encompass any carrier, which does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered. For example, for parenteral administration, the active protein(s) may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

The active ingredients of the pharmaceutical composition according to the invention can be administered to an individual in a variety of ways. The routes of administration include intradermal, transdermal (e.g. in slow release formulations), intramuscular, intraperitoneal, intravenous, subcutaneous, oral, epidural, topical, intrathecal, rectal, and intranasal routes. Any other therapeutically efficacious route of administration can be used, for example absorption through epithelial or endothelial tissues or by gene therapy wherein a DNA molecule encoding the active agent is administered to the patient (e.g. via a vector), which causes the active agent to be expressed and secreted in vivo. In addition, the protein(s) according to the invention can be administered together with other components of biologically active agents such as pharmaceutically acceptable surfactants, excipients, carriers, diluents and vehicles.

For parenteral (e.g. intravenous, subcutaneous, intramuscular) administration, the active protein(s) can be formulated as a solution, suspension, emulsion or lyophilised powder in association with a pharmaceutically acceptable parenteral vehicle (e.g. water, saline, dextrose solution) and additives that maintain isotonicity (e.g. mannitol) or chemical stability (e.g. preservatives and buffers). The formulation is sterilized by commonly used techniques.

The bioavailability of the active protein(s) according to the invention can also be ameliorated by using conjugation procedures which increase the half-life of the molecule in the human body, for example linking the molecule to polyethylenglycol, as described in the PCT Patent Application WO 92/13095.

The therapeutically effective amounts of the active protein(s) will be a function of many variables, including the type of protein, the affinity of the protein, any residual cytotoxic activity exhibited by the antagonists, the route of

administration, the clinical condition of the patient (including the desirability of maintaining a non-toxic level of endogenous clusterin activity).

A "therapeutically effective amount" is such that when administered, the clusterin exerts a beneficial effect on the peripheral neurological disease. The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including clusterin pharmacokinetic properties, the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Clusterin can preferably be used in an amount of about 0.001 to 10 mg/kg or about 0.01 to 5 mg/kg or body weight or about 0.1 to 3 mg/kg of body weight or about 1 to 2 mg/kg of body weight. Further preferred amounts of clusterin are amounts of about 0.1 to 1000 µg/kg of body weight or about 1 to 100 µg/kg of body weight or about 10 to 50 µg/kg of body weight

The route of administration, which is preferred according to the invention, is administration by subcutaneous route. Intramuscular administration is further preferred according to the invention.

In further preferred embodiments, clusterin is administered daily or every other day.

The daily doses are usually given in divided doses or in sustained release form effective to obtain the desired results. Second or subsequent administrations can be performed at a dosage which is the same, less than or greater than the initial or previous dose administered to the individual. A second or subsequent administration can be administered during or prior to onset of the disease.

According to the invention, clusterin can be administered prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, in particular with an interferon. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions.

The invention further relates to a method for treating a peripheral neurological disease comprising administering to a patient in need thereof an effective amount of clusterin, or of an agonist of clusterin activity, optionally together with a pharmaceutically acceptable carrier.

A method for treating a peripheral neurological disease comprising administering to a patient in need thereof an effective amount of clusterin, or of an

agonist of clusterin activity, and an interferon, optionally together with a pharmaceutically acceptable carrier, is also within the present invention.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent application, issued U.S. or foreign patents or any other references, are entirely incorporated by reference herein, including all data, tables, figures and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various application such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

Having now described the invention, it will be more readily understood by reference to the following examples that are provided by way of illustration and are not intended to be limiting of the present invention.

EXAMPLES

EXAMPLE 1: Recombinant expression of clusterin

The vector depicted in Fig. 2 was used for expression of recombinant murine his-tagged clusterin in HEK cells which was purified as follows:

The culture medium sample (100 ml) containing the recombinant protein with a C-terminal 6His tag was diluted with one volume cold buffer A (50 mM NaH_2PO_4; 600 mM NaCl; 8.7 % (w/v) glycerol, pH 7.5) to a final volume of 200 ml. The

sample was filtered through a 0.22 um sterile filter (Millipore, 500 ml filter unit) and kept at 4°C in a sterile square media bottle (Nalgene).

The purification was performed at 4°C on the VISION workstation (Applied Biosystems) connected to an automatic sample loader (Labomatic). The purification procedure was composed of two sequential steps, metal affinity chromatography on a Poros 20 MC (Applied Biosystems) column charged with Ni ions (4.6 x 50 mm, 0.83 ml), followed by gel filtration on a Sephadex G-25 medium (Amersham Pharmacia) column (1,0 x 10 cm).

For the first chromatography step the metal affinity column was regenerated with 30 column volumes of EDTA solution (100 mM EDTA; 1 M NaCl; pH 8.0), recharged with Ni ions through washing with 15 column volumes of a 100 mM $NiSO_4$ solution, washed with 10 column volumes of buffer A, followed by 7 column volumes of buffer B (50 mM NaH_2PO_4; 600 mM NaCl; 8.7 % (w/v) glycerol, 400 mM; imidazole, pH 7.5), and finally equilibrated with 15 column volumes of buffer A containing 15 mM imidazole. The sample was transferred, by the Labomatic sample loader, into a 200 ml sample loop and subsequently charged onto the Ni metal affinity column at a flow rate of 10 ml/min. The column was subsequently washed with 12 column volumes of buffer A, followed by 28 column volumes of buffer A containing 20 mM imidazole. During the 20 mM imidazole wash loosely attached contaminating proteins were elution of the column. The recombinant His-tagged protein was finally eluted with 10 column volumes of buffer B at a flow rate of 2 ml/min, and the eluted protein was collected in a 1.6 ml fraction.

For the second chromatography step, the Sephadex G-25 gel-filtration column was regenerated with 2 ml of buffer D (1.137 M NaCl; 2.7 mM KCl; 1.5 mM KH_2PO_4; 8 mM Na_2HPO_4; pH 7.2), and subsequently equilibrated with 4 column volumes of buffer C (137 mM NaCl; 2.7 mM KCl; 1.5 mM KH_2PO_4; 8 mM Na_2HPO_4; 20 % (w/v) glycerol; pH 7.4). The peak fraction eluted from the Ni-column was automatically through the integrated sample loader on the VISION loaded onto the Sephadex G-25 column and the protein was eluted with buffer C at a flow rate of 2 ml/min. The desalted sample was recovered in a 2.2 ml fraction. The fraction was filtered through a 0.22 um sterile centrifugation filter (Millipore), frozen and stored at –80C. An aliquot of the sample was analyzed on SDS-PAGE (4-12 % NuPAGE gel; Novex) by coomassie staining and Western blot with anti-His antibodies.

Coomassie staining. The NuPAGE gel was stained in a 0.1 % coomassie blue R250 staining solution (30 % methanol, 10 % acetic acid) at room temperature

for 1 h and subsequently destained in 20 % methanol, 7.5 % acetic acid until the background was clear and the protein bands clearly visible.

Western blot. Following the electrophoresis the proteins were electrotransferred from the gel to a nitrocellulose membran e at 290 mA for 1 hour at 4°C. The membrane was blocked with 5 % milk powder in buffer E (137 mM NaCl; 2.7 mM KCl; 1.5 mM KH_2PO_4; 8 mM Na_2HPO_4; 0.1 % Tween 20, pH 7.4) for 1 h at room temperature, and subsequently incubated with a mixture of 2 rabbit polyc lonal anti-His antibodies (G-18 and H-15, 0.2ug/ml each; Santa Cruz) in 2.5 % milk powder in buffer E overnight at 4°C. After further 1 hour incubation at room temperature, the membrane was washed with buffer E (3 x 10 min), and then incubated with a secondary HRP-conjugated anti-rabbit antibody (DAKO, HRP 0399) diluted 1/3000 in buffer E containing 2.5 % milk powder for 2 hours at room temperature. After washing with buffer E (3 x 10 minutes), the membrane was developed with the ECL kit (Amersham Pharmacia) for 1 min. The membrane was subsequently exposed to a Hyperfilm (Amersham Pharmacia), the film developed and the western blot image visually analysed.

Protein assay. The protein concentration was determined using the BCA protein assay kit (Pierce) with bovine serum albumin as standard. The average protein recovery was 216 µg purified clusterin per 100 ml culture medium.

Analysis of the purified protein in non-reducing SDS PAGE showed that the recombinant protein had the heterodimeric structure of native clusterin (not shown).

EXAMPLE 2: Protective effect of clusterin (AS900035) on neuropathy induced by sciatic nerve crush in mice

Abbreviations

CMAP : compound muscle action potential
EMG : electromyography
IGF-1 : insulin-like growth factor
i.p. : intraperitoneally
s.c. : subcutaneous
s.e.m. : standard error of the mean
vs : *versus*

Introduction

Neuropathies are usually selective as to the type of PNS neuron affected (e.g. sensory versus autonomic) and indeed also to the subtype of neurons (small versus large). Axotomy of peripheral nerves is the most commonly used animal model for appraising the neuroprotective effects of neurotrophic factors. Traumatic nerve injury, plexus lesions and root lesions are a serious complication of accidents. In addition, pressure on peripheral nerve that can cause myelin damage frequently seen in disorders such as carpal tunnel syndrome or is associated with spinal column orthopedic complications. Axotomy produces phenomena, like cell death, reduced axonal conduction velocity, and altered neurotransmitter levels in damaged neurons. Crush lesions allow for regeneration, an additional process of interest in relation to neuropathic states (McMahon. and Priestley, 1995).

A fundamental question in cellular neurobiology is the regulation of nerve regeneration after injury or disease. Functional nerve regeneration requires not only axonal sprouting and elongation, but also new myelin synthesis. Remyelination is necessary for the restoration of normal nerve conduction and for protection of axons from new neurodegenerative immunologic attacks. The primary goal of research in neurodegenerative disorders is ultimately to develop interventions that prevent neuronal death, maintain neuronal phenotype and repair neuronal and myelin damage. Many studies have been devoted to the unraveling of molecular and cellular mechanisms responsible for the complete regeneration of axotomized spinal motor neurons (Fawcett et al., 1990; Funakoshi et al., 1993). Injury-induced expression of neurotrophic factors and corresponding receptors may play an important role in the ability of nerve regeneration. Previous studies have shown a significant improvement of nerve regeneration with various peptides and non-peptides compounds like insulin-like growth factor (IGF -1), ACTH (Lewis et al., 1993; Strand et al., 1980), testosterone (Jones, 1993), SR 57746A (Fournier et al., 1993) and 4-Methylcatechol (Kaechi K et al. 1993, 1995; Hanaoka Y et al. 1992).

The present study was carried out to evaluate nerve regeneration in mice treated with clusterin at different doses. In this model a positive effect of clusterin on neuronal and axonal (sensory and motor neurons) survival and regeneration, on myelination or macrophage inflammation could lead to a restoration of motor function. The regeneration may be measured according to the restoration of sensorimotor functions and morphological studies. Therefore in the present work electrophysiological recordings and histomorphometric analysis were performed in parallel.

Materials and Methods

Animals

Seventy-two 8 weeks-old females C57bl/6 RJ mice (Elevage Janvier, Le Genest-St-Isle, France) were used. They were divided into 6 groups (n = 12): (a) vehicle sham operated group; (b) vehicle nerve crush operated group; (c) nerve crush/clusterin (300 µg/kg); (d) nerve crush/clusterin (1000 µg/kg); (e) nerve crush/4-methylcatechol (10 µg/kg); (f) nerve crush/osteopontin (100 µg/kg).

They were group-housed (12 animals per cage) and maintained in a room with controlled temperature (21-22°C) and a reversed light-dark cycle (12h/12h) with food and water available *ad libitum*. All experiments were carried out in accordance with institutional guidelines.

Lesion of the sciatic nerve

The animals were anaesthetized with i.p. injection of 60 mg/kg ketamine chlorhydrate (Imalgène 500®, Rhône Mérieux, Lyon, France). The right sciatic nerve was surgically exposed at mid thigh level and crushed at 5 mm proximal to the trifurcation of the sciatic nerve. The nerve was crushed twice for 30 s with a haemostatic forceps (width 1.5 mm; Koenig; Strasbourg; France) with a 90 degree rotation between each crush.

Planning of experiments and pharmacological treatment

Electromyographical (EMG) testing was performed once before the surgery day (baseline) and each week during 2 weeks following the operation.

The day of nerve crush surgery was considered as day (D) 0. No test was performed during the 4 days following the crush.

Body weight and survival rate were recorded every day.

From the day of nerve injury to the end of the study, clusterin or 4-methylcatechol was administered daily by intraperitoneal (i.p) route, whereas daily injection of osteopontin (AS900011) was performed subcutaneous (s.c.).

At the 2nd week, 4 animals per group were sacrificed and sciatic nerve was dissected to perform morphological analysis.

Electrophysiological recording

Electrophysiological recordings were performed using a Neuromatic 2000M electromyograph (EMG) (Dantec, Les Ulis, France). Mice were anaesthetized by

intraperitoneal injection of 100 mg/kg ketamine chlorhydrate (Imalgene 500®, Rhône Mérieux, Lyon, France). The normal body temperature was maintained at 30°C with a heating lamp and controlled by a contact thermometer (Quick, Bioblock Scientific, Illkirch, France) placed on the tail.

Compound muscle action potential (CMAP) was measured in the *gastrocnemius* muscle after a single 0.2 ms stimulation of the sciatic nerve at a supramaximal intensity (12.8 mA). The amplitude (mV), the latency (ms) and the duration (time needed for a depolarization and a repolarization session) of the action potential were measured. The amplitude is indicative of the number of active motor units, while the distal latency indirectly reflects motor nerve conduction and neuromuscular transmission velocities.

Morphometric analysis

Morphometric analysis was performed 2 weeks after the nerve crush. Four randomly selected animals per groups were used for this analysis. Mice were anesthetized with i.p. injection of 100 mg/kg Imalgène 500®. A 5 mm segment of sciatic nerve was excised for histology. The tissue was fixed overnight with a 4 % aqueous solution glutaraldehyde (Sigma, L'Isle d'Abeau-Chesnes, France) in phosphate buffer solution (pH = 7.4) and maintained in 30 % sucrose at 4°C until use. The nerve was fixed in 2 % osmium tetroxide (Sigma, L'Isle d'Abeau-Chesnes, France) in phosphate buffer for 2 hr and dehydrated in serial alcohol solutions and embedded in Epon. Embedded tissues were then placed at 70°C during 3 days for polymerisation. Transverse sections of 1.5 µm were made with a microtome and stained of 1% of toluidine blue (Sigma, L'Isle d'Abeau-Chesnes, France) for 2 min and dehydrated and mounted in Eukitt. Cross sections were obtained at the middle of the crush site. Morphometric analysis and fiber counts were performed on the total area of the nerve section using a semi-automated digital image analysis software (Biocom, France). The proportions of degenerating and non-degenerating myelinated fibers were analysed. Myelinated fibers showing multi-lobular axoplasm and/or irregular myelin sheath were considered as fibers undergoing processes of degeneration. The following parameters were calculated: axon area, myelin area and fiber area (axon and myelin area).

Data analysis

Global analysis of the data was performed using one factor or repeated measure analysis of variance (ANOVA) and one-way ANOVA, and non-parametric

tests (Mann Whitney test). Dunnett's test was used further when appropriate. The level of significance was set at $p < 0.05$. The results were expressed as mean ± standard error of the mean (s.e.m.).

Results

All animals survived after the nerve crush procedures. Throughout the study, several mice died: on day 2 (nerve crush/AS900011 n° 8 and nerve crush/AS900035 at 1 mg/kg n° 12); on day 7 (nerve crush/vehicle n° 9 and nerve crush/AS900035 at 1 mg/kg n° 9) due to the anesthetic.

Animal weight

As illustrated in Figure 3, all animals showed slight decrease in their body weight during 2-3 days following the surgery. Then, animals showed a progressive recovery of their body weight. The different treatments with clusterin (AS900035), did not induce any significant changes in the body weight of mice with crushed sciatic nerve when compared to untreated mice.

Electrophysiological measurements

Amplitude of the compound muscular action potential (Fig. 4):

In sham-operated animals, there was not significant change in the CMAP amplitude throughout the study. In contrast, crush of the sciatic nerve induced a dramatic decrease in the amplitude of CMAP with a decrease >90% at D7 and D14 when compared to the respective levels of sham-operated animals. When mice with crushed sciatic nerve were treated with clusterin, at 300 µg/kg or 1 mg/kg, or osteopontin at 100 µg/kg, they demonstrated a significant increase (about 1.5 times) in the CMAP amplitude as compared to the level in untreated mice. Similarly, 4-MC treatment also enhanced the CMAP amplitude of mice with nerve crush, but to a lesser extent than clusterin or osteopontin.

Latency of the compound muscular action potential (Fig 5.):

In sham-operated animals, there was no deterioration of CMAP latency throughout the study. In contrast, mice with crushed sciatic nerve showed 1.2 times greater CMAP latency than sham-operated animals. In mice with crushed sciatic nerve treated with clusterin or osteopontin, the CMAP latency value was significantly

reduced as compared to the one of untreated mice. At day 7, this effect could be observed after treatment with 0.3 mg/kg of clusterin and 0.1 mg/kg of osteoponti n. At day 14, both concentrations of clusterin were efficacious.

Duration of the compound muscular action potential (Fig. 6):

In sham-operated animals, the duration of CMAP was not statistically different to the baseline value. In contrast, mice with crus hed sciatic nerve showed a significant extension of CMAP duration, especially at D14 where the duration was 3 times greater than in sham-operated animals. When mice with crushed sciatic nerve were treated with clusterin at 300 µg/kg or osteopontin, they demonstrated a significantly reduced CMAP duration as compared to the vehicle treated animals with nerve crush.

Morphometric analysis

The morphometric analysis was carried out after termination of the experiment at day 14.

Percentage of degenerated (Fig. 7) and non-degenerated fibers (Fig. 8)

As shown in figure 7, the percentage of degenerated fibers in sciatic nerve of sham-operated animals (control) was < 20%. When the sciatic nerve was subjected to a crush, the proportion of degenerated fibers was signif icantly increased up to 60% (crush/vehicle). Treatment of mice with 300 µg/kg or 1mg/kg of clusterin induced a significant decrease in the proportion of degenerated fibers as compared to the untreated group.

Conversely, the proportion of non-degenerated fibers in sham-operated animals (control) was two times greater than in untreated mice with crushed sciatic nerve (crush/vehicle) (Figure 8). Treatment with clusterin at 300 µg/kg or 1mg/kg induced a significant increase in the density of non-degenerated fib ers.

Conclusions

The nerve-crush model is a very dramatic model of peripheral neuropathy. Immediately after the nerve crush most of the fibers having a big diameter are lost, due to the mechanical injury, leading to the strong decrease in the CMAP amplit ude. The CMAP latency is not immediately affected but shows an increase at 14 days due to additional degeneration of small diameter fibers by secondary, immune mediated degeneration (macrophages, granulocytes). The CMAP duration is

increased at day 7 and peaks at day 14. At 21 days (not shown), crush lesions allow for regeneration, an additional process of interest in relation to neuropathic states.

Clusterin showed a protective effect in the nerve crush model in mice on all parameters measured. Morphological studies performed 2 weeks post crush show a significant decrease in the percentage of degenerating fibers and an increase in total fiber number. Clusterin is as effective as the control molecule used in this study, 4-methylcatechol. This positive effect on functional and histological recovery may be due to clusterin effects on:

- direct protection of fibers from secondary immune mediated degeneration;
- accelerated remyelination and protection of axons;
- accelerated regeneration/ sprouting of damaged axons;
- increased myelin debris clean up by macrophages.
- modulation of macrophage response to axotomy.

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CLAIMS

1. Use of clusterin, an isoform, mutein, fused protein, functional derivative, active fraction, circularly permutated derivative, or salt thereof, or of an agonist of clusterin activity, for the manufacture of a medicament for the treatment and/or prevention of a peripheral neurological disease.

2. The use according to claim 1, wherein the peripheral neurological disease is selected from the group consisting of traumatic nerve injury of the peripheral nervous system (PNS), demyelinating diseases of the PNS, peripheral neuropathies and peripheral neurodegenerative diseases.

3. The use according to claim 1 or 2, wherein the peripheral neurological disease is caused by a congenital metabolic disorder.

4. The use according to any of the preceding claims, wherein the peripheral neurological disease is a peripheral neuropathy.

5. The use according to claim 4, wherein the peripheral neuropathy is diabetic neuropathy.

6. The use according to claim 4, wherein the peripheral neuropathy is chemotherapy-induced neuropathy.

7. The use according to any of the preceding claims, wherein the clusterin is selected from the group consisting of:

(a) A polypeptide comprising SEQ ID NO: 1;
(b) A polypeptide comprising amino acids 23 to 449 of SEQ ID NO: 1;
(c) A polypeptide comprising amino acids 35 to 449 of SEQ ID NO: 1;
(d) A polypeptide comprising amino acids 23 to 227 of SEQ ID NO: 1;
(e) A polypeptide comprising amino acids 35 to 227 of SEQ ID NO: 1;
(f) A polypeptide comprising amino acids 228 to 449 of SEQ ID NO: 1;
(g) A mutein of any of (a) to (f), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (f);

(h) A mutein of any of (a) to (f) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (f) under moderately stringent conditions or under highly stringent conditions;

(i) A mutein of any of (a) to (f) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (f);

(j) a salt or an isoform, fused protein, functional derivative, active fraction or circularly permutated derivative of any of (a) to (f).

8. The use according to claim 7, wherein the functional derivative comprises a PEG moiety.

9. The use according to claim 7 or 8, wherein the fused protein comprises an immunoglobulin (Ig) fusion.

10. The use according to any of the preceding claims, wherein the medicament further comprises an interferon and/or osteopontin, for simultaneous, sequential, or separate use.

11. The use according to claim 10, wherein the interferon is interferon -β.

12. The use according to any of the preceding claims, wherein the clusterin is used in an amount of about 0.001 to 100 mg/kg of body weight, or about 1 to 10 mg/kg of body weight, or about 5 mg/kg of body weight.

13. Use of a nucleic acid molecule for manufacture of a medicament for the treatment and/or prevention of a peripheral neurological disease, wherein the nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence selected from the group consisting of:

a) A polypeptide comprising SEQ ID NO: 1;

b) A polypeptide comprising amino acids 23 to 449 of SEQ ID NO: 1;

c) A polypeptide comprising amino acids 35 to 449 of SEQ ID NO: 1;

d) A polypeptide comprising amino acids 23 to 227 of SEQ ID NO: 1;

e) A polypeptide comprising amino acids 35 to 227 of SEQ ID NO: 1;

f) A polypeptide comprising amino acids 228 to 449 of SEQ ID NO: 1;

g) A mutein of any of (a) to (f), wherein the amino acid sequence has at least 40 % or 50 % or 60 % or 70 % or 80 % or 90 % identity to at least one of the sequences in (a) to (e);

h) A mutein of any of (a) to (f) which is encoded by a DNA sequence which hybridizes to the complement of the native DNA sequence encoding any of (a) to (f) under moderately stringent conditions or under highly stringent conditions;

i) A mutein of any of (a) to (f) wherein any changes in the amino acid sequence are conservative amino acid substitutions to the amino acid sequences in (a) to (f); or an isoform, fused protein, functional derivative, active fraction or circularly permutated derivative of any of (a) to (f).

14. The use according to claim 13, wherein the nucleic acid molecule further comprises an expression vector sequence.

15. The use of a vector for inducing and/or enhancing the endogenous production of clusterin, or an agonist of clusterin activity, in a cell in the manufacture of a medicament for the treatment and/or prevention of a peripheral neurological disease.

16. The use according to any of claims 13 to 15 for gene therapy.

17. Use of a cell that has been genetically modified to produce clusterin, or an agonist of clusterin activity, in the manufacture of a medicament for the treatment and/or prevention of a peripheral neurological disease.

18. A pharmaceutical composition comprising clusterin, or an agonist of clusterin activity, and an interferon, optionally together with one or more pharmaceutically acceptable excipients, for treatment and/or prevention of a peripheral neurological disease.

19. A pharmaceutical composition comprising clusterin, or an agonist of clusterin activity, and osteopontin, optionally together with one or more pharmaceutically acceptable excipients, for treatment and/or prevention of a peripheral neurological disease.

20. A method for treating a peripheral neurological disease comprising administering to a patient in need thereof an effective amount of clusterin, or of an agonist of clusterin activity, optionally together with a pharmaceutically acceptable carrier.

21. A method for treating a peripheral neurological disease comprising administering to a patient in need thereof an effective amount of clusterin, or of an agonist of clusterin activity, and an interferon, optionally together with a pharmaceutically acceptable carrier.

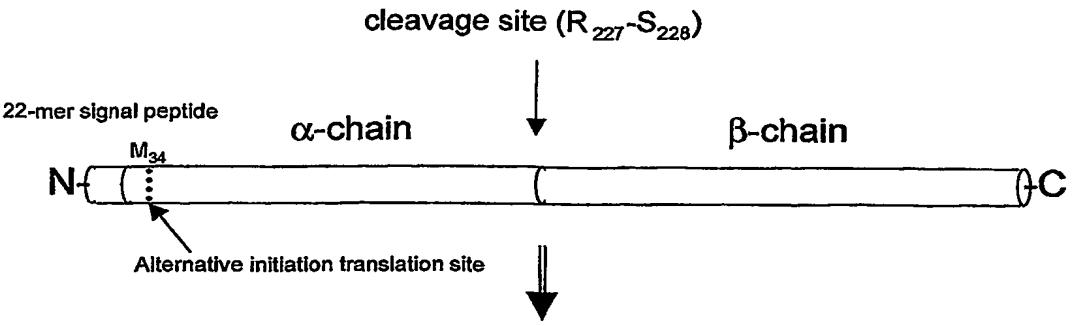
22. A method for treating a peripheral neurological disease comprising administering to a patient in need thereof an effective amount of clusterin, or of an agonist of clusterin activity, and osteopontin, optionally together with a pharmaceutically acceptable carrier.

ABSTRACT

The invention relates to the use of clusterin, or of an agonist of clusterin activity, for treatment or prevention of peripheral neurological diseases.

The invention further relates to the use of a combination of clusterin and interferon, or of a combination of clusterin and osteopontin, for treatment or prevention of peripheral neurological disease.

A



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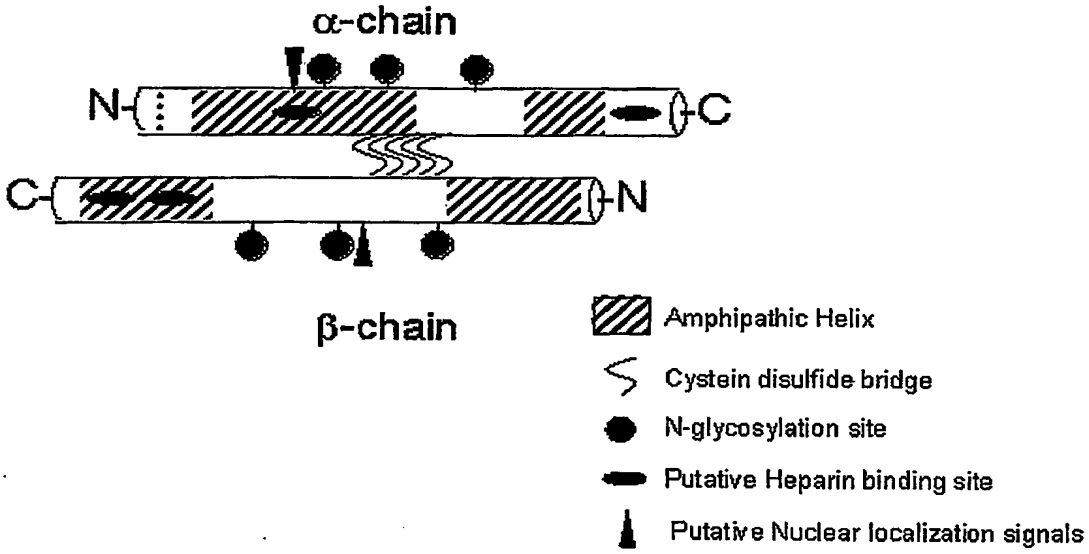

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Fig. 1

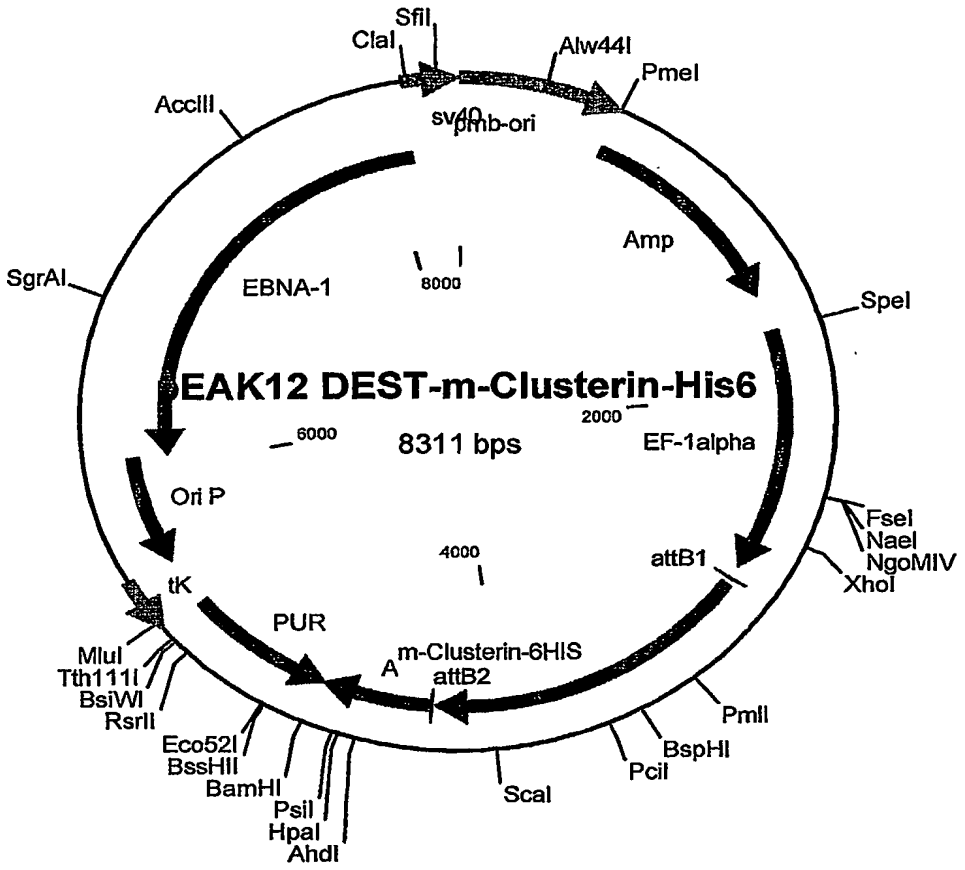
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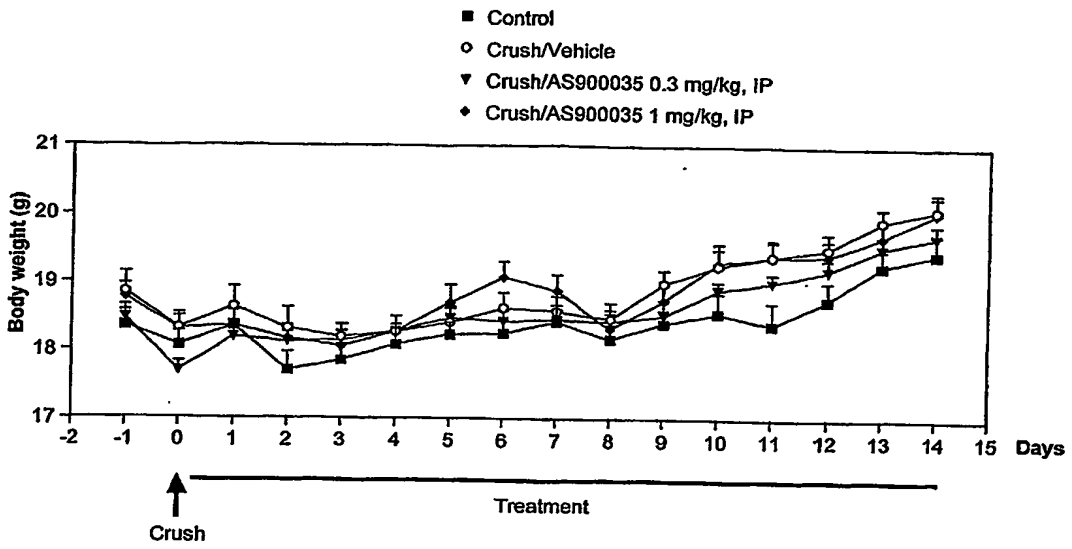
Fig. 2


Control
Crush/Vehicle
Crush/AS900035 0.3 mg/kg, IP
Crush/AS900035 1 mg/kg, IP
Body weight (g)
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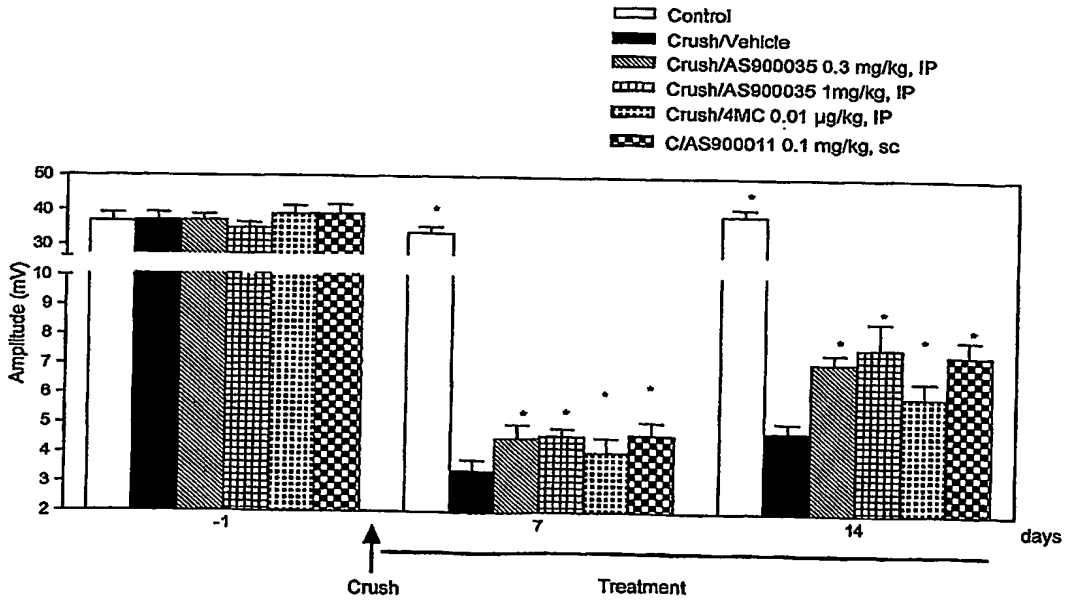
20
19
18
17
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 Days
Crush
Treatment

Fig. 3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Crush/Vehicle
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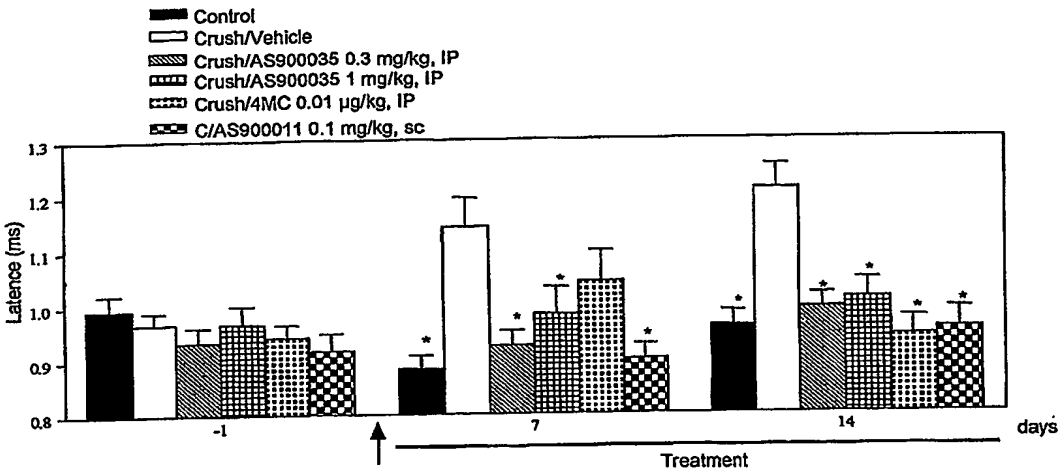
Crush/AS900035 1mg/kg, IP
Crush/4MC 0.01 µg/kg, IP
C/AS900011 0.1 mg/kg, sc
Amplitude (mV)
60
40
30
10
9
8
7
6
5
4
3
2
-1
7
14
days
Crush
Treatment

Fig. 4


Control
Crush/Vehicle
Crush/AS900035 0.3 mg/kg, IP
Crush/AS900035 1 mg/kg, IP
Crush/4MC 0.01 µg/kg, IP
C/AS900011 0.1 mg/kg, sc
Latence (ms)
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1.2
1.1
1.0
0.9
0.8
-1
7
14
days
Treatment

Fig. 5

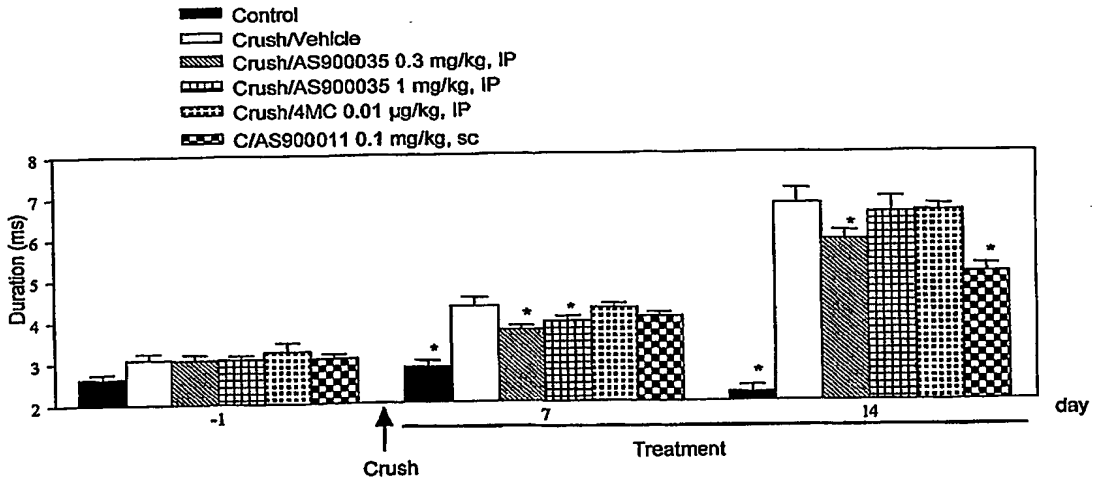
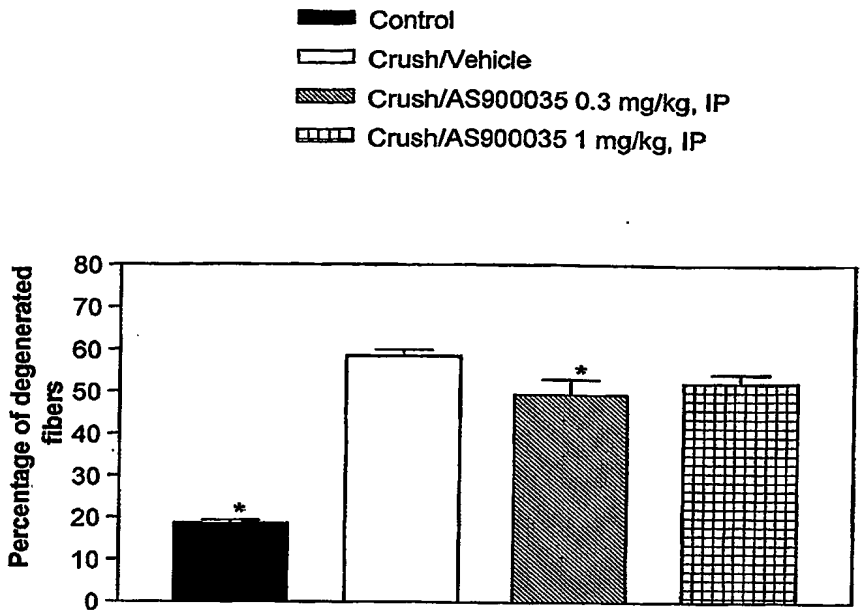

Control
Crush/Vehicle
Crush/AS900035 0.3 mg/kg, IP
Crush/AS900035 1 mg/kg, IP
Crush/4MC 0.01 µg/kg, IP
C/AS900011 0.1 mg/kg, sc
Duration (ms)
8
7
6
5
4
3
2
-1
7
14
day
Treatment
Crush

Fig. 6


Control
Crush/Vehicle
Crush/AS900035 0.3 mg/kg, IP
Crush/AS900035 1 mg/kg, IP
Percentage of degenerated fibers
80
70
60
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40
30
20
10
0
*
*

Fig. 7

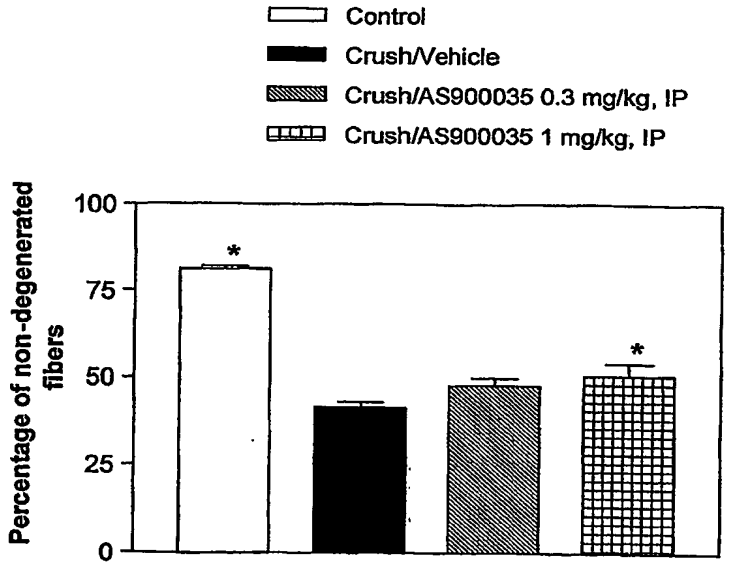
Control
Crush/Vehicle
Crush/AS900035 0.3 mg/kg, IP
Crush/AS900035 1 mg/kg, IP
Percentage of non-degenerated fibers
100
75
50
25
0
*
*

Fig. 8

SEQUENCE LISTING

<110> Applied Research Systems ARS Holding N.V.

<120> USE OF CLUSTERIN FOR THE TREATMENT AND/OR PREVENTION OF PERIPHERAL NEUROLOGICAL DISEASES

<130> 830 EP/EPA

<160> 1

<170> PatentIn version 3.1

<210> 1
<211> 449
<212> PRT
<213> homo sapiens

<400> 1

Met Met Lys Thr Leu Leu Leu Phe Val Gly Leu Leu Leu Thr Trp Glu
1 5 10 15

Ser Gly Gln Val Leu Gly Asp Gln Thr Val Ser Asp Asn Glu Leu Gln
20 25 30

Glu Met Ser Asn Gln Gly Ser Lys Tyr Val Asn Lys Glu Ile Gln Asn
35 40 45

Ala Val Asn Gly Val Lys Gln Ile Lys Thr Leu Ile Glu Lys Thr Asn
50 55 60

Glu Glu Arg Lys Thr Leu Leu Ser Asn Leu Glu Glu Ala Lys Lys Lys
65 70 75 80

Lys Glu Asp Ala Leu Asn Glu Thr Arg Glu Ser Glu Thr Lys Leu Lys
85 90 95

Glu Leu Pro Gly Val Cys Asn Glu Thr Met Met Ala Leu Trp Glu Glu
100 105 110

Cys Lys Pro Cys Leu Lys Gln Thr Cys Met Lys Phe Tyr Ala Arg Val
115 120 125

Cys Arg Ser Gly Ser Gly Leu Val Gly Arg Gln Leu Glu Glu Phe Leu
130 135 140

Asn Gln Ser Ser Pro Phe Tyr Phe Trp Met Asn Gly Asp Arg Ile Asp
145 150 155 160

Ser Leu Leu Glu Asn Asp Arg Gln Gln Thr His Met Leu Asp Val Met
165 170 175

Gln Asp His Phe Ser Arg Ala Ser Ser Ile Ile Asp Glu Leu Phe Gln
180 185 190

Asp Arg Phe Phe Thr Arg Glu Pro Gln Asp Thr Tyr His Tyr Leu Pro
195 200 205

Phe Ser Leu Pro His Arg Arg Pro His Phe Phe Phe Pro Lys Ser Arg
210 215 220

Ile Val Arg Ser Leu Met Pro Phe Ser Pro Tyr Glu Pro Leu Asn Phe
225 230 235 240

His Ala Met Phe Gln Pro Phe Leu Glu Met Ile His Glu Ala Gln Gln
245 250 255

Ala Met Asp Ile His Phe His Ser Pro Ala Phe Gln His Pro Pro Thr
260 265 270

Glu Phe Ile Arg Glu Gly Asp Asp Asp Arg Thr Val Cys Arg Glu Ile
275 280 285

Arg His Asn Ser Thr Gly Cys Leu Arg Met Lys Asp Gln Cys Asp Lys
290 295 300

Cys Arg Glu Ile Leu Ser Val Asp Cys Ser Thr Asn Asn Pro Ser Gln
305 310 315 320

Ala Lys Leu Arg Arg Glu Leu Asp Glu Ser Leu Gln Val Ala Glu Arg
325 330 335

Leu Thr Arg Lys Tyr Asn Glu Leu Leu Lys Ser Tyr Gln Trp Lys Met
340 345 350

Leu Asn Thr Ser Ser Leu Leu Glu Gln Leu Asn Glu Gln Phe Asn Trp
355 360 365

Val Ser Arg Leu Ala Asn Leu Thr Gln Gly Glu Asp Gln Tyr Tyr Leu
370 375 380

Arg Val Thr Thr Val Ala Ser His Thr Ser Asp Ser Asp Val Pro Ser
385 390 395 400

Gly Val Thr Glu Val Val Val Lys Leu Phe Asp Ser Asp Pro Ile Thr
405 410 415

Val Thr Val Pro Val Glu Val Ser Arg Lys Asn Pro Lys Phe Met Glu
420 425 430

Thr Val Ala Glu Lys Ala Leu Gln Glu Tyr Arg Lys Lys His Arg Glu
435 440 445

Glu